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ABSTRACTS

1

Inotodiol ameliorates Th2 mediated airway inflammation by attenuating mast cell activities in a mouse model of asthma.

Sabin Acharya, Liu Ye, Rema Naskar, Inkyu Hwang.
phrsabin@gmail.com

Immunology and Immunopharmacology Laboratory, College of Pharmacy, Chungnam National University, 99 Daekak-ro Yuseong-gu, Daejeon 34134, Republic of Korea

Inotodiol is a lanostane triterpenoid found only in Chaga mushroom. In a previous study, we have shown that inotodiol holds an activity to suppress the mast cell function *in vivo* and ease allergy symptoms observed in a chicken ovalbumin (cOVA)-induced mouse model of food allergy. Based on that, we hypothesized that inotodiol could exert a therapeutic effect on Th2-mediated allergic asthma where mast cell also plays a critical role and examined the effect with a mouse model of allergic asthma. For inducing asthmatic symptoms, Balb/c mice were sensitized with cOVA on days 1 and 14 (i.p.), followed by challenges with aerosolized cOVA on days 23, 24, 25, 26, 27, 30, and 31. Groups of mice were treated with inotodiol by oral gavage for the last five days of challenges at either 2 or 6 mg per kg body weight (mpk). Twenty-four hours after the last challenge, respiratory mechanics were evaluated with the forced oscillation technique (flexivent). Inotodiol-treated mice (6 mpk) showed a significant improvement in the overall respiratory resistance and the total elastance in response to methacholine challenges. The inflammatory lesions and the goblet cell hyperplasia in the airway mucosa were also found to be reduced by the inotodiol treatments. Supporting the idea that the anti-asthmatic effect of inotodiol is attributed to the inhibition of mast cell function, the levels of mast cell-specific protease-1 (MCPT-1) in bronchoalveolar lavage (BAL) fluid of inotodiol-treated mice not only were lowered by the treatments in a dose-dependent manner but also showed a strong correlation with the severities of the asthmatic symptoms. Additionally, the numbers of mast cells populating in the airway mucosa were also markedly decreased following the inotodiol-treatments. Together, it is indicated that inotodiol can be developed as a new effective and safe oral medication for the treatment of Th2-mediated eosinophilic asthma whose symptoms are often instigated by the activation of mast cells.

2

Anti-IgE vaccination prevents human IgE-mediated severe anaphylaxis in humanized mice

Eva Conde[1, 2, 3], Marija Backovic[4], Julien Stackowicz[1, 2], Ophélie Godon[1], Samir Hamdi[3], Bernard Malissen[6], Frédéric Fiore[6], Vincent Serra[3], Pierre Bruhns[1, *], Laurent L. Reber[1, 5, *].
laurent.reber@inserm.fr; eva.conde-garcia@inserm.fr

[1] Unit of Antibodies in Therapy and Pathology, Institut Pasteur, UMR 1222 INSERM, F-75015 Paris, France. [2] Sorbonne Université, ED394, F-75005 Paris, France
[3] Neovacs SA, F-75014 Paris, France. [4] Unit of Structural Virology, Institut Pasteur, UMR 3569 CNRS, F-75015 Paris, France. [5] Toulouse Institute for Infectious and Inflammatory Diseases, Infinity. UMR 1291 INSERM, Toulouse, France. [6] Centre d'Immunophénomique, Aix Marseille Université, INSERM, CNRS, F-13288 Marseille, France. [*]equal contribution.

Anaphylaxis is the most dramatic clinical manifestation of allergy. In allergic subjects, the allergen is recognized by allergen-specific IgE bound to the receptor FcεRI on mast cells, which promotes degranulation of these cells and the release of mediators, including histamine. We developed a vaccine strategy against IgE to induce long-term protection from IgE-mediated mast cell activation. A fragment of human IgE was used to generate a conjugate vaccine technology termed kinoid. To assess the efficacy of IgE vaccination, we generated a mouse model humanized for IgE and its high-affinity receptor FcεRI. IgE vaccination induced long-term production of anti-human IgE neutralizing antibodies without any detectable adverse effect. It reduced both circulating and FcεRI-bound human IgE, and protected against severe IgE-mediated anaphylaxis in IgE/FcεRI humanized mice. Thus, IgE vaccination represents a promising, cost-effective, long-term therapeutic strategy for the treatment of IgE-mediated anaphylaxis, and other IgE- and mast cell-driven allergic conditions.

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Targeting mast-cell plasticity re-shapes the tumor microenvironment of melanoma

Caterina Iuliano 1, Kaesler.S1, Biedermann.T1
1Technische Universität München, Department of Dermatology and Allergy, 80802 Munich, Germany
caterina.iuliano@tum.de

Background: Resistance to therapy in malignant melanoma is linked to an increase of phenotypic heterogeneity of melanoma cells to their response to pro-inflammatory signals from the tumor microenvironment (TME). Within the TME of melanoma, an accumulation of mast cells (MC) has been found. In previous work we were able to show that targeting tumor-resident MCs leads to an effective anti-melanoma immune response. Since MCs are very plastic, we are currently investigating how different stimuli activate MCs and how this (might) affect the TME and the control of melanoma.
Results: Through stimulation with IL-33, bone marrow-derived MCs (BMMCs) were activated to produce a wide range of cytokines that could be detected in the supernatant. This strong activation also impacts the transcription of genes, as shown by RNA-Sequencing. In combinations with other stimuli antagonistically or synergistically effects could be generated, indicating that the plasticity of MCs can be manipulated; a process which can be blocked by adding resveratrol. Upon the highest concentration of detected cytokines in the supernatant were chemokines that play a crucial role in the recruitment of immune cells, such as CCL3 and CCL22. In vivo experiments of wild type mice show that IL-33 does not impact tumor size, however alters the immune cell recruitment to the tumor. The impact of MCs in the control of melanoma is yet to be deciphered.

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Distinct lipid mediator release and contraction by MRGPRX2 agonist and antigen in guinea pig trachea

Jielu Liu[1], Johan Kolmert[1], Jesper Säfholm[1], Anna-Karin Johnsson[1], Javier Zurita[2], Craig E. Wheelock[2], Sven-Erik Dahlén[1], Gunnar Nilsson[3], and Mikael Adner[1]
jielu.liu@ki.se

[1]Experimental Asthma and Allergy Research Unit, Institute of Environmental Medicine (IMM), Karolinska Institutet, Stockholm, Sweden.
[2]Division of Physiological Chemistry 2, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
[3]Division of Immunology and Allergy, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden and Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Background: Mast cells can be activated by allergen aggregating Fc-receptors and non-immunological agents such as compound 48/80 (C48/80) acting through the Mas-related G-protein coupled receptor member X2 (MRGPRX2). The aim of this study was to compare the bronchoconstriction and release of mediators after activation of guinea pig tracheal segments by C48/80 or antigen.
Methods: Contractions and release of histamine and lipid mediators were studied in organ baths containing tracheal rings from naïve or house dust mite (HDM) sensitized guinea pigs. Mediators were measured in the bath fluid by ELISA and LC-MS/MS.
Results: C48/80 and HDM caused concentration dependent contractions (E_{max}: 52±6% and 85±3% respectively). Submaximal bolus dose of C48/80 (150 µg/mL) and HDM (0.1 µg/mL) induced similar contractions (E_{max}: approximately 40%). These were inhibited by histamine H_1 receptor antagonist mepyramine (1 µM) during the initial 15 minutes, whereas only the combination of mepyramine (1 µM) and 5-lipoxygenase (5-LOX) activating protein inhibitor MK886 (10 µM) dampened the entire 60 minutes response to C48/80 and HDM. Both C48/80 (500 µg/mL) and HDM (10 µg/mL) increased bath fluid levels of histamine and 15 lipid mediators including prostaglandin D_2 metabolites. Leukotriene E_4 was only increased after HDM challenge, whereas C48/80 increased release of 13 additional mediators originating from multiple biosynthetic pathways.
Conclusion: Both C48/80 and HDM induced strong smooth muscle contraction through the release of histamine and 5-LOX products. The difference in lipid mediator release for C48/80 and HDM supports activation of distinct biosynthesis routes, possibly explained by activation of different mast cell phenotypes.

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Multifunctional fluorescently labeled polymer-coated GdF_3 nanoparticles inhibit degranulation in rat basophilic leukemia (RBL) cells

Vadym Sulimenko[1], Oleksandr Shapoval[2], Anastasiya Klebanovych[1], Mariia Rabyk[2], Pavlo Shapoval[3], Ondřej Kaman[4], Marcela Filipová[2], Eliška Rydvalová[2], Pavel Dráber[1], Daniel Horák[2]
sulimen@img.cas.cz

[1] Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Heyrovského nám. 2, 162 06 Prague 6, Czech Republic
[2] Laboratory of Biology of Cytoskeleton, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, CZ 142 20 Prague 4, Czech Republic
[3] National University "Lvivska Polytechnika", Department of Physical, Analytical and General Chemistry, Sv.Yura square 9, 79013, Lviv, Ukraine
[4] Institute of Physics of the Czech Academy of Sciences, Cukrovarnická 112/10, 162 00 Prague 6, Czech Republic

Antigen-mediated activation of mast cells and basophils initiates degranulation and release of inflammatory mediators. Aggregation of membrane-associated high-affinity IgE receptors (FcεRIs) triggers activation pathway causing transient phosphorylation of proteins from signaling cascades that leads to Ca^{2+} influx across the plasma membrane, and release of preformed mediators from granules. Activation by FcεRI aggregation is accompanied with reorganization of cytoskeleton and changes in cell morphology and enhanced cell adhesion and migration. Rare-earth fluoride nanoparticles have recently attracted a great deal of attention due to their unique electronic, chemical, and optical characteristics and good chemical stability. In particular, lanthanide fluoride particles are finding interesting applications in X-ray therapy and magnetic resonance imaging (MRI). The interaction of metal-based nanoparticles with mast cells and basophils is emerging new field in the study of signaling and cellular responses during activation events. In the present work, we prepared GdF_3 nanoparticles modified with a fluorescently labeled polymer (GdF_3@PSSMA-PSDA-A488). This biocompatible nanoparticles bind to cell surface of rat basophilic leukemia (RBL) cells and interfere with signaling pathway initiated by crosslinking of FcεRIs by multivalent antigen. Nanoparticles suppress sustained Ca^{2+} mobilization and degranulation measured by β-hexosaminidase release. Functionalized nanoparticles could thus represent new tool for the study of signaling pathways during mast cell activation and for development of new strategies for the treatment of allergic diseases.

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Repurposing of the antiepileptic drug levetiracetam to restrain adenocarcinoma and neuroendocrine prostate cancer

Roberta Sulsenti[1*], Barbara Frossi[2*], Lucia Bongiovanni[3], Valeria Cancila[3], Paola Ostano[4], Irene Fischetti[1], Claudia Enriquez[1], Laura Botti[1], Francesca Guana[4], Giovanna Chiorino[4], Claudio Tripodo[3], Carlo Pucillo[2], Mario P. Colombo[1] and Elena Jachetti[1]
roberta.sulsenti@istitutotumori.mi.it

[1] Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[2] Immunology Section, Department of Medicine, University of Udine, Udine, Italy
[3] Tumor Immunology Unit, Department of Health Sciences, University of Palermo, Italy
[4] Laboratory of Cancer Genomics, Fondazione Edo ed Elvo Tempia, via Malta 3 - 13900 Biella, Italy
*These authors contributed equally

Castration-resistant prostate cancers (CRPC) often evolve into fatal neuroendocrine (NEPC) tumors in resistance to androgen deprivation and/or anti-androgen therapy. Drugs effective against both CRPC and NEPC are needed. We previously described a dual role of mast cell (MCs) in promoting adenocarcinoma but also in preventing NEPC. This finding suggests that targeting both MCs and NEPC cells could be effective against prostate cancer. Using an *in silico* drug repurposing approach, we identified the antiepileptic drug levetiracetam as a potential candidate for this purpose. Levetiracetam targets SV2A protein, that we found at high levels in NEPC cells and MCs infiltrating prostate adenocarcinoma but negliglible in adenocarcinoma cells. *In vitro*, levetiracetam inhibited the proliferation of NEPC cells and the degranulation of MCs. In mice bearing subcutaneous tumors it was partially active on both NEPC and adenocarcinoma, the latter effect due to inhibition of MC-derived MMP9. In surgical castrated TRAMP mice, levetiracetam reduced the onset and frequency of both adenocarcinoma and NEPC. Our results demonstrate that levetiracetam can directly restrain NEPC development after androgen deprivation, and that it can also block adenocarcinoma progression through the inhibition of MCs functions. These findings open the possibility to test levetiracetam in prostate cancer and in MC-mediated diseases.